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Mineral nutrition in radish (Raphanus sativus L. 'Cherry Belle') grown with nitrification inhibitors.

Margie Lynn Stratton
University of Massachusetts Amherst

MINERAL NUTRITION IN RADISH (RAPHANUS SATIVUS L. 'CHERRY BELLE') GROWN WITH NITRIFICATION INHIBITORS

A Thesis Presented

by

MARGIE LYNN STRATTON

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Approved as to style and content by:

Allen V. Barker, Chairman of Committee

John H. Baker, Committee Member

William A. Rosenau, Committee Member

Allen V. Barker, Department Head

DEDICATION

This thesis is a labor of love dedicated to my Messiah, my Mother, my Model, my Mentor, and my Mirror; you all know who you are.

SPECIAL RECOGNITION

Special thanks and warmest wishes are extended to Betty Bradley, Mary Flanigan, Ina Gestick, Athena Morris, Carol Neves, Diane Tarlin, Jayne Timmerman, and Carol White who as women supported my endeavor; Dr. Jean English, Dr. Bernie Rubinstein, Dr. Richard Damon, Dr. Oliver Zajicek, Dr. William Rosenau, and Alfred Boicourt who inspired me as a scientist; Dr. Allen V. Barker, Dr. John H. Baker, and Dr. Haim B. Gunner who kept me on the right track. I aspire to that kind of excellence. And a special thanks to Dr. William Rosenau for all his "eleventh hour" assistance. Many thanks to the manufacturers of the nitrification inhibitors for their generous donation of materials; and to the staff and the professors of the Plant and Soil Sciences Department for all their instruction and assistance. My deepest gratitude and appreciation are extended to the people who made this thesis possible: my parents, my friends, my teachers, and of course, Douglas.

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INTRODUCTION

The use of nitrogen in crop production is a major concern of agronomists and horticulturists. Nitrogen is an important constituent of plants, comprising two to four percent of the plant dry matter. It is found in such life - sustaining molecules as proteins, nucleic acids, hormones, enzymes and coenzymes.

An insufficient nitrogen supply to a plant produces characteristic deficiency symptoms, the evidence of metabolic disturbances. Plants grown without adequate nitrogen will be spindly and small with chlorotic leaf tissues, due to destruction of chloroplasts and restricted chloroplast development (Thomson and Weier, 1971). Deficiency of nitrogen depresses synthesis and translocation of cytokinins in plants (Wagner and Michael, 1961. This action may promote premature aging of the plant.

To ensure that adequate nitrogen is supplied to corps, fertilizers are used. Animal manure has been used as a fertilizer since ancient times (Huxley, 1978). Commercial fertilizers are modern carriers of nitrogen dating back to about 1840, but with wide spread usage starting after World War II. Today, over eleven million tons of nitrogen are consumed in fertilizers in the United States (Agricultural Statistics, 1981). Ammoniacal fertilizers (anhydrous ammonia,

urea, ammonium nitrate, ammonium sulfate) make up the vast majority of the total tonnage of nitrogen fertilizers consumed in American agriculture.

Although the initial source of nitrogen fertilizer may be ammoniacal, nitrifying organisms in the soil convert the ammonium - N into Nitrate - N; therefore, the primary nutrient source of nitrogen to plants is nitrate ions (Figure 1).

Because plants have evolved in an environment in which nitrate is the dominant nitrogen ion, plants have little tolerance of ammonium nutrition and exhibit toxic responses when ammonium ions are abundant in the medium supporting growth (Barker and Mills, 1980). Unlike ammonium ions, nitrate ions are not bound to negatively-charged soil colloids. Leaching losses of nitrates from the soil may be severe (Goring, 1961) and flexibility with respect to time of application of nitrogen, such as fall application, is lost.

Denitrification, a process which does not occur with ammonia causes substantial losses of nitrate from the soil (Broadbent and Clark, 1965). Organic fertilizers which mimic organic carriers are employed to govern the flow of nitrogen from fertilizer to plants and to conserve on the losses which are characteristic of nitrate fertilizers (Maynard and Lorenz, 1980, Barker and Mills, 1980). The combined losses of nitrogen from leaching and denitrification may equal or exceed 25% of nitrogen applied in fertilizers (Huber et al, 1977; Broadbent and Clark, 1965).

Figure 1. Partial presentation of the Soil Nitrogen Cycle

Biological Processes

A. Mineralization (ammonification)
B. Nitrification
C. Ion Uptake
D. Nitrogen Fixation
E. Nitrogen Immobilization
F. Denitrification
G. Humification
H. Nitrate Reduction
I. Dissolution

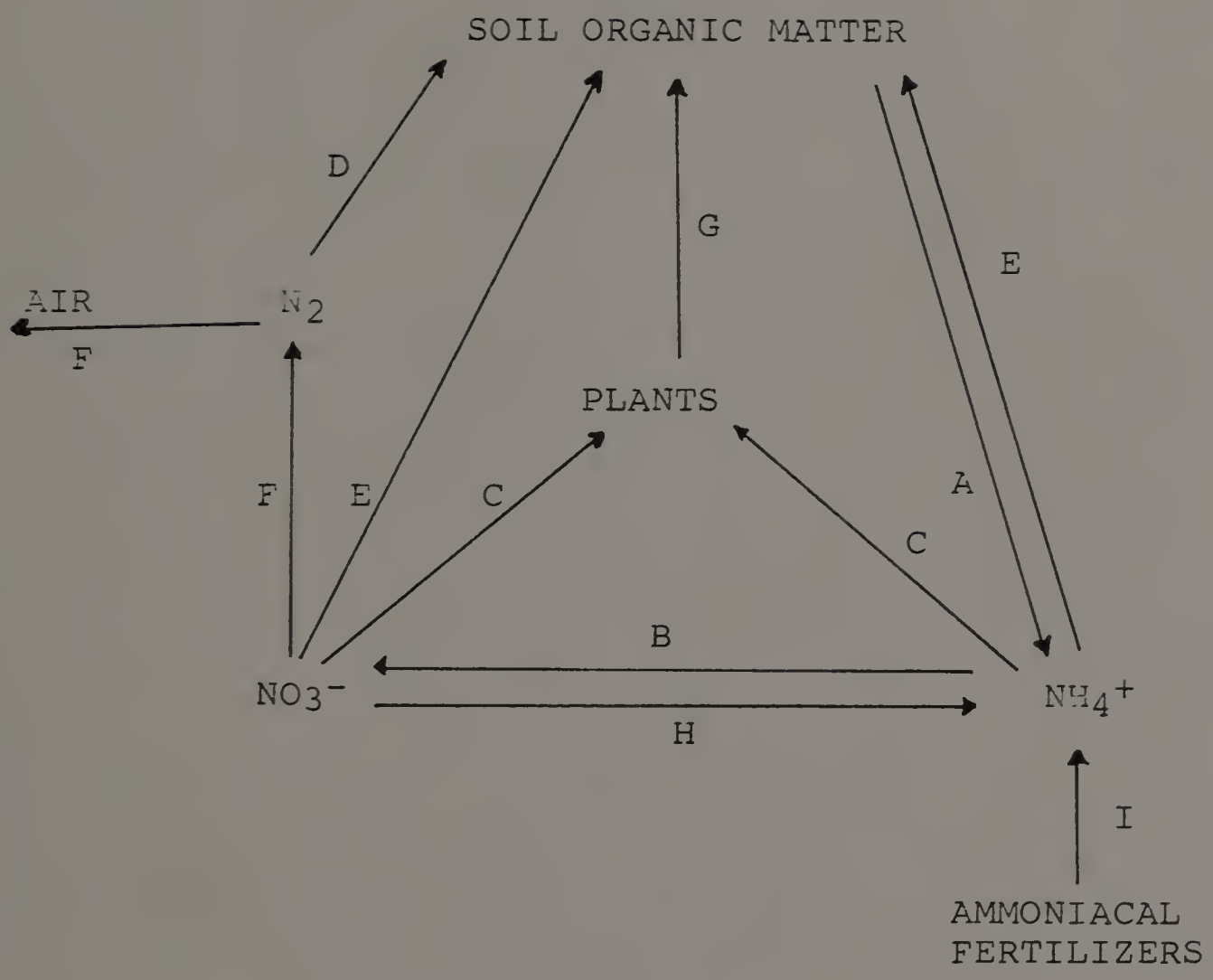

SOIL ORGANIC MATTER
D
G
E
AIR
N_2
F
PLANTS
F
E
C
C
A
B
NO_3^-
NH_4^+
H
I
AMMONIACAL
FERTILIZERS

Figure 1

Recommendations and practices of fertilization have been adjusted for the relatively low recovery of fertilizer nitrogen by crops. Rates of application are in excess of crop removal to compensate for these losses, or split applications of nitrogen are used to increase efficiency of recovery. The manufacture of nitrogen fertilizers is energy-intensive and consumes much natural gas. About one-third of the energy consumption in agriculture is through nitrogen fertilizers (Stout, 1979). Splitting the application of nitrogen consumes fuel, time and machinery. Each of these practices, increased fertilization and split applications, are financially expensive to the farmer.

Another hazard from excessive nitrates in the root zone, other than the losses of nitrogen to ground water, is that plants will absorb nitrates in luxury amounts. Nitrate accumulation in vegetables and in forage crops has been a matter of considerable concern in public and animal health. Excess nitrate nutrition in humans may manifest itself as methemoglobinemia when ingested by young infants or by other susceptible individuals (Maynard et al., 1976). Excess nitrates in forage crops used as feed have caused concern in the poisoning of farm animals (Wright and Davidson, 1964). For these reasons it is desirable to limit excess nitrate application and to inhibit nitrification when ammonia fertilizers are applied.

A variety of compounds, notably fungicides, exhibit properties which inhibit nitrification (Bundy and Bremmer, 1973, 1974, Hughes and Welch, 1970). N-Serve is an effective chemical for this purpose as it is quite specific in the inhibition of Nitrosomonas spp. (Goring, 1962). N-Serve has been indicated an an inhibitor of dentrification (Cribbs and Mills, 1979).

Mills et al. (1973) noted that N-Serve was toxic to young plants of bean, corn, cucumber, peas, and pumpkin. English et al. (1980) reported that N-Serve reduced the concentrations of calcium and magnesium in corn. Kowal and Barker (1981) noted the same effects with N-Serve applied to cabbage which received ammoniacal or organic fertilizers. Torrey et al. (1982) noted the same effects with radish and associated some of the difficulties in plant growth to the accumulation of ammonium - N in the medium for growth.

The toxic reactions from N-Serve and the adverse interactions of N-Serve and organic or ammoniacal fertilizers has stimulated this study which is designed to determine if other nitrification inhibitors exhibit the same reactions and interactions which diminish plant growth and affect mineral content of vegetables.

C H A P T E R II

MATERIALS AND METHODS

Common nitrification inhibitors were chosen to compare and contrast with N-Serve. In the first experiment five inhibitors were compared (Table 1).

Different amounts of each inhibitor were applied as shown in Table 2. Five replications of each treatment were included. All pots were supplied with organic nitrogen in the form of processed sludge (Milorganite, Milwaukee Sewerage Commission). The analysis of Milorganite is 6% nitrogen, 2%,phosphorus and no potassium (6:2:0). Nitrogen was supplied at the rate of 800 mg of N per pot, each pot containing 1200 g of soil.

The Milorganite, the inhibitor, and the soil were weighed out for each pot in each treatment. The soil used was seven parts Hadley fine sandy loam, three parts shredded peat, two parts sand by volume and was unsterilized. The Milorganite, inhibitor, and soil were mixed together in a five gallon cement mixer for five minutes. After mixing, the treated soil was weighed into five six-inch azalea pots, making the replications for each treatment. Every treatment was prepared in this manner with the exception of Extend, which is a liquid preparation; the other four inhibitors were in granular form and mixed into the soil easily.

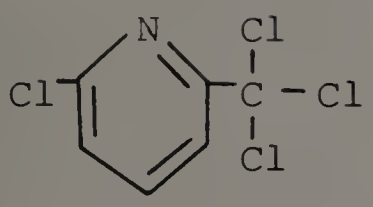
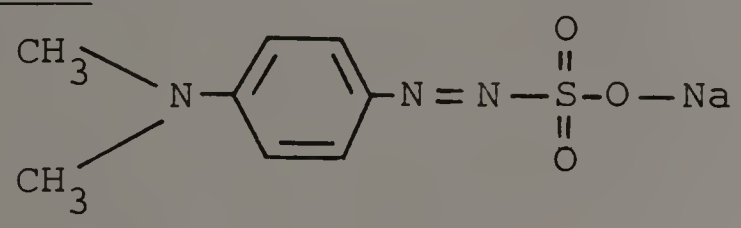
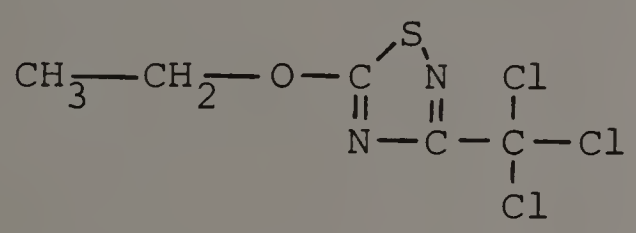
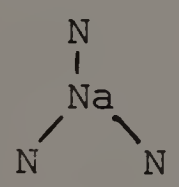
N-Serve 	2-Chloro-6-(trichloromethyl) pyridine) N-Serve is a trademark of the Dow Chemical Company, USA.
Dexon 	P-(Dimethylamino)benzenediazo sodium sulfonate Dexon is a trademark of the Chemagro Corporation, Kansas City, Missouri.
Truban 	5-Ethoxy-3-trichloromethyl-1,2, 4-thiadiazole Truban is a trademark of Mallinckrodt, Inc., St. Louis, Missouri.
Sodium azide 	
Extend Polyhydric alcohol Aliphatic solvents Aromatic sulfonate Alkylene ether sulfate	Extend is a trademark of Kalo Laboratories, Kansas City, Missouri.

Table 2. Active Ingredients of Some Nitrification Inhibitors

PPM Active Ingredient

Control	N-Serve	Truban	Dexon	Extend	Sodium Azide
0	5	5	5	2.16	5
	10	10	10	4.33	10
	20	20	20	8.65	20
	40	40	40	17.30	40
		80	80		

Table 2. Active Ingredient levels of nitrification inhibitors applied in Experiment One.

Extend, a liquid, was particularly difficult to mix into the soil as a treatment. To overcome expected difficulties in uniformity of mixing I first treated a stock mixture of 1000g of soil by pouring the Extend on the stock soil and allowing it to evaporate for 36 hours. As a carrier solvent I used toluene to facilitate thorough distribution. This stock mixture was used to formulate all the Extend treatments. Due to lack of information concerning Extend I calculated the treatment concentrations incorrectly. When information became available I recalculated the treatment concentrations and found them to be 2.2, 4.3, 8.7 and 17.4 ppm rather than 5, 10, 20 and 40 ppm as originally designed.

The soil for all treatments was seeded with 20 radish seeds per pot on 25 September 1979. The radish used was Raphanus sativus L. 'Cherry Belle'. It was necessary to repeatedly seed the highest levels of sodium azide treatments due to failure to germinate. The plantings were thinned over a three-week period to a population of five seedlings per pot. All pots were watered regularly and were assembled in five randomized blocks in a greenhouse. All plants were grown for six weeks from date of seeding. After six weeks of growth, photographic slides were made. At this point the plants were harvested using a clean razor blade to sever the true roots from the reddened edible portion and to separate the reddened portion from the green foliage along the line of color change. The true roots were discarded, and the reddened portions were

called "roots". The foliage was termed "shoots". Upon harvest, fresh weights of both roots and shoots were recorded. All roots and shoots were rinsed quickly and thoroughly, first in tap water and then twice in de-ionized water. After rinsing, the roots and shoots were blotted thoroughly on paper towels.

The radish roots and shoots were dried in an oven for one week with air circulating at a temperature of 83°C. Dry weights were then recorded, and the plant tissue was pulverized by hand using a porcelain mortar and pestle.

Having been dried and ground, the plant tissue was weighed into samples for wet ashing. The wet ashing procedure which I used follows. One hundred milligram samples of dried, ground, plant material were weighed, and each sample was put into a 50-ml Erlenmeyer flask. Five to seven milliliters of concentrated reagent grade nitric acid were added to each flask using an automatic pipette. The flasks were heated on a hot plate at intermediate heat until the evolution of brown fumes subsided. Then reagent grade hydrogen peroxide was added dropwise until all brown fumes disappeared. The flasks were further heated until the brown fumes reappeared, and hydrogen peroxide was again added dropwise until all brown fumes disappeared. The heating and adding of hydrogen peroxide continued alternately for four cycles until brown fumes no longer evolved from the flasks. At this point the organic matter had been oxidized, and after cooling at room temperature, the

solutions appeared clear.

When the solutions reached 22°C, they were transferred quantitatively from each Erlenmeyer flask to 25-ml volumetric flasks. The samples were then brought up to volume, and appropriate dilutions were made to facilitate spectrophotometric analysis. A Perkin-Elmer 306 atomic absorption spectrophotometer was used to analyze the wet-ashed samples for magnesium, calcium, potassium, and sodium.

Nitrate analysis of shoot tissue was used an in indicator of nitrification inhibition. I weighed samples of 400 mg each and put each sample into a 125-ml Erlenmeyer flask. To each flask, I added 50 ml of 0.01M KH_2PO_4. Then the flasks were shaken for ten minutes by a wrist action shaker, and the resulting suspensions were filtered into beakers using Whatman Paper #31. The solutions were analyzed for nitrates electrometrically (Barker, 1974). Thus analysis of the first experiment included magnesium, calcium, potassium, sodium, and nitrate content of radish shoot and root tissue.

Two subsequent experiments were designed and were conducted one year later, in the fall of 1980. The crop was 'Cherry Belle' radish, as in Experiment One, and the same soil and soil mixing procedures were used. I designed these experiments to study the effects of different nitrogen sources and nitrification inhibitors on the mineral nutrition of radish. Experiment number two was designed using one nitrogen source and three granular nitrification inhibitors at four levels of concentration.

N-Serve, Dexon and Truban were used, Extend and sodium azide were not. Extend was not used because of difficulty of application mentioned previously, and sodium azide was not used because it appeared to be toxic under the conditions of Experiment One.

Amount of nitrification inhibitor applied was determined using one of two methods. For N-Serve, I determined that 20 ppm was sufficient to produce significant visual symptoms and to affect the mineral nutrition of the radish crop. I used experimentally determined levels of toxicity for N-Serve as parameters for development of an upper limit (Torrey et al., 1982).

Twenty ppm N-Serve was identified as 100% treatment level with other levels of N-Serve treatment being set at 0%, 25%, 50% and 75% of that upper limit (see Table 3).

Likewise, "100% treatment levels" were identified for Truban and Dexon. The recommended rates of applications as they appear in the package directions were the "100% treatment levels" for Truban and Dexon. As with N-Serve 0%, 25%, 50% and 75% rates were included as treatment levels. Table 3 displays treatment levels used in Experiment Two.

As in Experiment One, 1200 g of treated, mixed soil were weighed into each six-inch azalea pot, and five randomized blocks were assembled in the same greenhouse. All experiments were conducted in September and October of 1979 and 1980.

In the second experiment, I seeded the 'Cherry Belle'

Relative Level	Level of Application		
	N-Serve	Truban	Dexon
	ppm		
0%	—	—	—
25%	5	25	60
50%	10	50	120
75%	15	75	180
100%	20	100	240

Table 3. Treatment levels in Experiment Two

radishes on 6 September 1980 and gradually thinned the plants to five per pot. The radishes were watered regularly and allowed to grow for six weeks.

Upon harvest the plants were handled as in Experiment One. I photographed them, harvested them, weighed roots and shoots, and rinsed the plants as before. After quickly blotting the plants, I placed them in the drying oven for one week. After oven-drying, the radish roots and shoots were weighed, and the shoots were ground in a Wiley mill using a 30-mesh screen. The dried, ground shoot tissue was wet ashed and analyzed spectrophotometrically for calcium, magnesium, and potassium and manganese. I did not analyze for sodium as I saw no difference in sodium levels in shoots or roots of radish grown in Experiment One. I did not analyze the roots for minerals as there were no significant differences in the mineral content of the roots in Experiment One.

To determine if nitrification had occurred, I analyzed the soil for nitrates and ammonia. Soil samples were taken on the date of harvest using a trowel to thoroughly mix the soil in each pot. Then soil samples were placed in poly-ethylene bags and sealed with twist-ties. These samples were frozen at -25°C and kept frozen until analysis.

Prior to nitrate analysis of the soil samples two 10-gram soil samples of each pot were weighed, one for analysis and the other for a moisture determination. For nitrate analysis the freshly thawed 10-gram soil samples were shaken for 10

minutes in 50 ml of 0.01 M KH_2PO_4 and filtered into beakers using Whatman #31 filter paper. These solutions were measured electrometrically for nitrate.

At this time a third set of freshly thawed 10-gram samples was subjected to analysis for ammonium. First the samples were shaken in 50 ml of 2M KCl solution for thirty minutes. I then filtered each suspension into Kjehdahl flasks using Whatman #31 filter paper and placed the flasks in position on a standard steam distillation apparatus after 2 ml of 50% NaOH were added. The escaping steam was condensed and collected in a 125-ml flask in which 10 ml of mixed boric acid indicator solution had been placed (Barker and Volk, 1964). The resulting distilled liquid was collected for five minutes after the indicator had turned from purple to green. These samples were then carefully back-titrated with potassium biiodate to the end point.

These procedures are also used for the analysis of tissue and soil in my third and final experiment. The third experiment was designed to study the effects of various nitrogen sources and nitrification inhibitors upon the mineral nutrition of radish. As in my first two experiments, the soil was seven parts Hadley fine sandy loam, three parts peat and two parts sand. This mix was unsterilized, as before. The inhibitor, N-Serve at 10 ppm, and the organic nitrogen source, Milorganite, were weighed and mixed into the soil using the cement mixer reserved for soil mixing. The treated

soil was weighed into six-inch azalea pots, 1200 g of soil in each pot. Five randomized blocks were assembled in the greenhouse. 'Cherry Belle' radish was seeded into the pots on 25 September 1980, and the plants were gradually thinned to five per pot.

The organic nitrogen source, Milorganite, was added prior to seeding. The ammonium sulfate and potassium nitrate were added as solutions after seeding. These solutions were applied in dilute form to prevent possible toxicity and osmotic pressure effects. Each treatment of inorganic nitrogen was applied in 100 ml doses, each containing 200 mg of nitrogen. The solution treatments were applied every three days as needed to attain the levels of nitrogen seen in Table 4. The solution treatments were started on day six after seeding, and the schedule of application can be seen in Table 5.

The radishes were then grown until they were six weeks old. Halfway through this period, I used an auger to take soil samples and immediately froze the samples in polyethylene bags with twist-ties. I also collected soil samples at harvest time and froze them for later analysis. Photographic slides of the radish plants were made one week before harvest. Upon harvest, I weighed the roots and shoots and rinsed and blotted them quickly, as in the two previous experiments, and placed them in the same drying oven as previously used. The tissue was dried for one week at 82^{o}C,

Without N-Serve	With 10 ppm Active Ingredient N-Serve		
nitrogen supplied as $(NH_4)_2SO_4$	nitrogen supplied as $(NH_4)_2SO_4$	nitrogen supplied as KNO_3	nitrogen supplied as Milorganite
200 mg N/pot	200 mg N/pot	200 mg N/pot	200 mg N/pot
400 mg N/pot	400 mg N/pot	400 mg N/pot	400 mg N/pot
600 mg N/pot	600 mg N/pot	600 mg N/pot	600 mg N/pot
800 mg N/pot	800 mg N/pot	800 mg N/pot	800 mg N/pot

Table 4. Experiment Design, Experiment Three

		Treatments receiving 200 mg N doses		
		$(NH_4)_2SO_4$		KNO_3
1) First Application	Sept. 30, 1980 Day six after seeding	with N-Serve	without N-Serve	with N-Serve
		200 mg N	200 mg N	200 mg N
		400 mg N	400 mg N	400 mg N
		600 mg N	600 mg N	600 mg N
		800 mg N	800 mg N	800 mg N
2) Second Application	Oct. 3, 1980 Day nine after seeding	400 mg N	400 mg N	400 mg N
		600 mg N	600 mg N	600 mg N
		800 mg N	800 mg N	800 mg N
3) Third Application	Oct. 6, 1980 Day twelve after seeding	600 mg N	600 mg N	600 mg N
		800 mg N	800 mg N	800 mg N
4) Fourth Application	Oct. 9, 1980 Day fifteen after seeding	800 mg N	800 mg N	800 mg N

Table 5. Schedule of 200 mg N solution Treatments in Experiment Three

and dry weights were recorded. Dried foliage tissue was ground in a Wiley Mill using a 30-mesh screen, and the ground tissue was wet ashed using the procedure described for the first experiment. I determined magnesium, calcium, and potassium contents in the radish foliage using the same spectrophotometric method I used in Experiments One and Two.

To determine if nitrification and mineralization occurred I analyzed the soil samples for ammonium using the distillation procedure previously described.

Straight line regression analyses of data were preformed (Steel and Torrie, 1960).

C H A P T E R III

RESULTS

Experiment One

In my first experiment I studied the effects of five nitrification inhibitors on the mineral nutrition and weight of radish roots and shoots. The results can be seen in Tables 6 and 7. As concentrations of N-Serve and Truban increased to 40 and 80 ppm respectively, fresh and dry weights of roots decreased significantly (P=0.01). Fresh and dry weights of radish roots did not change significantly when concentrations of Dexon or Extend were increased to 80 or 17.3 ppm, respectively. With increased concentration of sodium azide (greater than 10 ppm) several plants died, and many were smaller than the control plants.

An increase in inhibitor concentration up to levels used in Experiment One did not change calcium or magnesium concentrations in the radish roots. At higher levels of N-Serve and Truban (20 ppm for both), roots showed an increase in potassium content, but increasing levels of Dexon, Extend and sodium azide did not change potassium content of radish roots.

Fresh and dry weights of radish shoots decreased with increased levels of N-Serve, Truban and sodium azide (5, 10 and 20 ppm, respectively). Higher concentrations of

Inhibitor	ppm Active Ingredient	Roots: Fresh Weight	Roots: Dry Weight	Roots: Ca	Roots: Mg	Roots: K
		g/pot	g/pot	dry weight basis	dry weight basis	dry weight basis
N-Serve	5	19	1.2	0.27	0.33	4.25
	10	13	0.9	0.23	0.29	4.25
	20	3	0.2	0.26	0.25	5.76
	40	1	0.05	0.31	0.28	6.69
Truban	5	28	1.7	0.21	0.26	3.44
	10	15	0.9	0.20	0.27	3.95
	20	5	0.3	0.21	0.21	5.15
	40	8	0.5	0.19	0.22	4.49
	80	7	0.4	0.19	0.21	4.83
Dexon	5	30	1.8	0.27	0.36	4.01
	10	35	2.0	0.26	0.33	4.46
	20	32	1.8	0.26	0.32	4.01
	40	34	1.9	0.26	0.39	4.86
	80	22	1.3	0.28	0.35	4.26
Extend	2.16	36	1.9	0.29	0.34	4.23
	4.32	35	2.0	0.29	0.32	4.21
	8.65	36	2.0	0.29	0.33	3.93
	17.30	38	2.1	0.26	0.31	4.19
Sodium azide	5	38	2.1	0.27	0.31	4.66
	10	34	1.9	0.28	0.32	4.05
	20	23	1.3	0.25	0.30	4.49
	40	7	0.5	0.19	0.27	5.41
Control	0	39	2.36	0.27	0.31	3.89

Table 6. Results of Root Analysis, Experiment One

Inhibitor	ppm Active Ingredient	Shoots: Fresh Weight	Dry Weight	NO_3	Ca	Mg	K
		-----g/pot-----		--% dry weight basis--			
N-Serve	5	24	1.9	1.22	2.70	0.85	3.49
	10	20	1.5	1.01	2.17	0.77	4.10
	20	15	1.1	0.28	1.27	0.52	4.79
	40	12	1.0	0.18	1.13	0.51	4.85
Truban	5	31	2.5	0.63	2.26	0.70	2.62
	10	27	1.9	0.59	1.98	0.71	3.43
	20	22	2.0	0.17	1.18	0.58	4.75
	40	22	1.9	0.17	1.17	0.60	4.64
	80	20	1.8	0.14	1.05	0.58	4.18
Dexon	5	27	2.0	1.10	3.37	1.13	2.12
	10	26	1.9	1.01	3.61	1.15	1.77
	20	24	2.0	1.03	3.27	1.13	2.25
	40	28	2.1	1.13	3.17	1.10	2.18
	80	22	1.9	1.18	2.83	0.95	2.75
Extend	2.16	29	2.3	1.10	3.73	1.13	2.29
	4.32	28	2.2	1.17	3.65	1.11	2.67
	8.65	33	2.3	0.99	3.95	1.16	2.35
	17.30	30	2.2	1.02	3.71	1.15	2.45
Sodium azide	5	33	2.4	1.02	3.81	1.19	2.59
	10	29	2.2	1.03	3.51	1.13	2.94
	20	24	1.9	1.24	2.95	0.95	3.87
	40	13	1.1	0.93	2.25	0.80	5.09
Control	0	28	2.2	0.81	3.95	1.13	1.91

Table 7. Results of Shoot Analysis, Experiment One

N-Serve and Truban and higher (20 ppm) concentrations of sodium azide resulted in decreased levels of calcium and magnesium in the radish shoots, and all levels of N-Serve, Truban and sodium azide resulted in increased levels of potassium relative to the control. (The control shoots contained an average of 1.9%K.) Increasing levels of Dexon or Extend resulted in no change in calcium, magnesium, potassium or nitrate-N concentrations in radish shoots. Nitrate-N concentration in radish shoots was also unaffected by increasing levels of sodium azide. However, a significant decrease in nitrate-N concentration in radish shoots was realized when increasing levels of N-Serve and Truban were applied ($P=0.01$). Twenty ppm N-Serve and Truban resulted in 0.28 and 0.17% NO_3-N, respectively, compared to 0.80% NO_3-N in control shoots.

Experiment Two

In the second experiment, higher levels of Dexon and Truban were applied (up to 240 and 100 ppm, respectively, compared to 80 ppm applied in Experiment One). These higher applications did not change the dry weight of shoots or roots from the lowest levels of Dexon and Truban application (60 and 25 ppm, respectively) to the highest levels of inhibitor applied. However, fresh and dry weights of roots and shoots at all levels of Truban in Experiment Two were

significantly lower than the control (P=0.01), so that an effect on tissue weights was seen at all concentrations of inhibitor. The fresh and dry weights of roots, and dry weights of shoots were lower than the control when any level of Dexon was applied in Experiment Two. At all levels of N-Serve, roots and shoots decreased in fresh and dry weights (P=0.01) as compared to the control (see Table 8). Calcium and magnesium concentrations in shoots were significantly lower than those of the control when N-Serve or Truban was applied (P=0.01). The highest levels of Dexon lowered calcium and magnesium concentration in shoots (P=0.01). Potassium levels of shoots were increased significantly (P=0.01) when N-Serve or Truban was applied, but application of Dexon resulted in only a slight increase of potassium concentration in shoots. All shoots of plants grown with inhibitor contained about 0.05% manganese compared to 0.02% in shoots grown with no inhibitor.

Soil ammonium was influenced greatly by the application of N-Serve, Truban, and Dexon. Truban-treated soil showed the largest increase of soil ammonium, ranging from 8 times the control amount (16.8 mg/pot) to 10 times that amount. Increases in ammonium in soil treated with N-Serve ranged from twice the control amount to 8 times the amount of ammonium found in the soil with no inhibitor. Soil ammonium in soil treated with Dexon ranged from 2 times the amount of ammonium in soil with no inhibitor (16.8 mg/pot) to 4 times that amount.

	Relative Treatment Level	ppm Active Ingredient	--Roots-- Fresh Wt. --g/pot--	Dry Wt. --g/pot--	----Soil------ ----mg/pot---- NH_4	NO_3
N-Serve	25%	5	12.6	0.79	30.6	182.1
	50%	10	6.8	0.44	112.2	159.9
	75%	15	1.7	0.06	157.8	147.5
	100%	20	3.4	0.25	142.2	121.1
Truban	25%	25	1.8	0.15	135.6	113.4
	50%	50	1.9	0.17	163.8	96.1
	75%	75	1.9	0.12	176.4	89.0
	100%	100	1.6	0.13	170.4	95.1
Dexon	25%	60	42.6	2.45	33.3	118.2
	50%	120	42.2	2.41	40.5	115.6
	75%	180	47.3	2.48	54.0	113.7
	100%	240	43.3	2.30	63.6	116.7
Control	0	0	55.8	3.38	16.8	187.1

	Relative Treatment Level	ppm Active Ingredient	-Shoots-- Fresh Wt. --g/pot--	Dry Wt. --g/pot--	-------Shoots-------- ----% dry wt. basis-- Ca	Mg	K	Mn
N-Serve	25%	5	27	2.1	0.86	0.44	2.35	0.05
	50%	10	22	1.7	0.70	0.45	2.83	0.05
	75%	15	12	0.9	0.66	0.41	3.12	0.05
	100%	20	20	1.5	0.65	0.49	2.85	0.04
Truban	25%	25	14	1.2	0.84	0.53	2.37	0.05
	50%	50	17	1.3	0.68	0.47	2.19	0.06
	75%	75	15	1.2	0.71	0.52	2.42	0.04
	100%	100	16	1.2	0.71	0.54	2.33	0.05
Dexon	25%	60	37	2.6	2.61	1.25	1.19	0.04
	50%	120	33	2.5	2.79	1.17	1.17	0.04
	75%	180	43	2.9	2.13	0.93	1.38	0.06
	100%	240	38	2.7	2.01	0.80	1.63	0.02
Control	0	0	42	3.5	3.39	1.44	1.00	0.02

Table 8. Results of Experiment Two

Soil NO_3-N was significantly lower in soils with inhibitor added (P=0.01). With no inhibitor added, the soil contained 187/mg pot nitrare-N. All levels of Dexon application (60 to 240 ppm) resulted in soil NO_3-N contents of about 115 mg/pot. Ten ppm N-Serve lowered soil NO_3-N levels to 160 mg/ pot and 20 ppm N-Serve lowered soil nitra-televels to 121 mg NO_3/pot and 100 ppm Truban applied resulted in 95 mg NO_3/pot. Photographic slides were made at six weeks (see Illustrations 2 and 3).

Experiment Three

In the third experiment, increases in all nitrogen sources resulted in significant decreases in fresh and dry weights of roots and shoots with the exception that there was no significant change in shoot weights when the nitrogen supplied as potassium nitrate was supplied at levels as high as 800 mg N/pot. Calcium concentrations in shoots were reduced by the highest applications of all nitrogen sources except potassium nitrate, and calcium concentrations were particularly low in the shoots of radishes grown with the highest levels of ammonium sulfate in the presence of N-Serve. (0.88%Ca compared to 1.97%Ca in the radish shoots grown at the same level of ammonium sulfate but no N-Serve). The higher levels of ammonium sulfate applied in the presence of N-Serve resulted in decreased levels of magnesium.

Illustration 2. Appearance of radish foliage grown in soil with 20 ppm N-Serve

Illustration 3. Appearance of radish foliage grown in soil with 20 ppm N-Serve, 100 ppm Truban and 280 ppm Dexon. Control plants are those at top center.

Magnesium levels were significantly higher in shoots grown in soil with ammonium sulfate and no N-Serve than in shoots grown in soil with both ammonium sulfate and 10 ppm N-Serve (P=0.01).

As nitrogen application increased from 200 mg/pot to 800 mg/pot in treatments with potassium nitrate and 10 ppm N-Serve, potassium content of radish foliage ranged from 3.6 to 6.9%K, and from 2.5 to 4.4%K for plants grown with Milorganite and 10 ppm N-Serve. Potassium concentration in shoots grown with 200 to 800 mg nitrogen supplied as ammonium sulfate, and no N-Serve ranged from 0.81 to 1.39% potassium.

Soil samples taken at three weeks showed equal amounts of ammonium for the soil with ammonium sulfate and no N-Serve, and the treatment with Milorganite and 10 ppm N-Serve. Soil treated with ammonium sulfate and N-Serve had the highest levels of ammonium. The analysis showed lower ammonium levels at six weeks than at three weeks and the soil with ammonium sulfate and no N-Serve exhibited a substantial decrease in soil ammonium over the last 3 weeks of the experiment. The soils with Miloganite and 10 ppm N-Serve contained almost twice as much ammonium as the soil with ammonium sulfate and no N-Serve at the six week analysis. However these soil ammonium levels were equal at three weeks.

All soil treated with potassium nitrate and N-Serve showed only trace amounts of ammonium even when nitrogen was supplied at the 800 mg level.

N-Source	mg N added	N-Serve ppm	---Roots--- Fresh Weight ---g/pot---	Dry Weight	------Soil---------- ------mg/pot-------- NH_4^+-N 3 WKS	NH_4^+-N 6WKS
$(NH)_4SO_4$	200	0	43	2.9	11	5
	400		34	2.2	61	6
	600		16	1.0	148	26
	800		4	0.3	229	84
$(NH)_4SO_4$	200	10	21	1.4	49	18
	400		2	0.3	198	125
	600		2	0.1	276	186
	800		1	0.04	328	268
KNO_3	200	10	59	3.0	tr	tr
	400		68	3.1	tr	tr
	600		40	2.1	tr	tr
	800		32	1.8	tr	tr
Milorganite	200	10	11	0.7	32	23
	400		2	0.1	100	69
	600		3	0.2	167	76
	800		1	0.08	221	135

N-Source	mg N added	N-Serve ppm	----Shoots--- Fresh Weight ----g/pot-----	Dry Weight	Shoots % dry weight basis Ca	Mg	K
$(NH)_4SO_4$	200	0	24	2.2	4.52	0.86	0.81
	400		36	2.8	4.05	0.83	0.98
	600		31	2.7	2.76	0.61	1.12
	800		16	1.6	1.97	0.41	1.39
$(NH)_4SO_4$	200	10	24	1.9	2.98	0.80	1.12
	400		13	1.0	1.60	0.56	1.82
	600		12	1.0	1.34	0.33	2.01
	800		3	0.3	0.88	0.25	2.08
KNO_3	200	10	25	1.8	3,79	0.50	3.59
	400		28	2.0	3.53	0.39	5.36
	600		25	1.9	3.12	0.33	6.11
	800		22	1.8	2.89	0.26	6.94
Milorganite	200	10	12	0.8	3.84	1.05	2.46
	400		8	0.4	2.74	0.73	2.91
	600		9	0.5	2.14	0.54	3.66
	800		8	0.5	1.99	0.36	4.40

Table 9. Results of Experiment Three

C H A P T E R IV

DISCUSSION

Two of the five compounds studied were shown to be effective nitrification inhibitors using nitrate-N concentration of radish shoots as an indicator of nitrification inhibition. Truban and N-Serve were effective inhibitors, and Dexon, Extend and sodium azide were not under the conditions of Experiment One. However, when Truban and Dexon were applied in concentrations recommended for general fungicidal activity, and soil ammonium and soil nitrate assays were used as an indication of nitrification inhibition, N-Serve, Truban and Dexon were all considered nitrification inhibitors.

Extend may prove to be an effective nitrification inhibitor when applied as intended, in a band with an ammonium type fertilizer, however it was ineffective under conditions at experiment one. Sodium azide does not inhibit nitrification effectively as indicated by the foliage nitrate assay. However sodium azide decreased calcium and magnesium and increased potassium content in the radish shoots. At first, the mineral effects may appear to be a result of the use of nitrification inhibitors as other researchers have noted similar effects on the mineral content of other crops such as bean, corn, and cabbage (Mills et al., 1973, English et al., 1980, Kowal and Barker, 1981). However, general growth

radishes was poor in soil with sodium azide added. Most plants died before harvest, and all pots containing sodium azide required repeated seeding before emergence occurred. The general condition of the plants was poor: roots thin and undeveloped, shoots chlorotic, and many pots empty. Under the conditions of Experiment One, sodium azide was toxic to 'Cherry Belle' radish, and Extend did not inhibit nitrification. For these reasons I chose not to work with sodium azide or Extend after the first experiment.

Dexon also was considered ineffective as a nitrification inhibitor under the conditions of Experiment One. Five to 80 ppm active ingredient of Dexon were applied in the first experiment. This was probably too low to study the effects of the active ingredient of Dexon, as recommended rate of application for general fungicidal activity is 280 ppm according to label instructions. In Experiment Two, I raised the level of Dexon to accomodate this upper limit.

In the second experiment I also applied that level of active ingredient of Truban that was indicated on the label as being an effective concentration for general fungicidal activity (100 ppm as compared with 80 ppm active ingredient applied in Experiment One). Under the conditions of Experiment Two, N-Serve, Dexon and Truban inhibited nitrification as indicated by soil ammonia and soil nitrate-N assays.

Application of these nitrification inhibitors increased potassium and decreased calcium and magnesium concentrations

in the radish shoots while decreasing fresh and dry weights of roots and shoots. The mineral effects may appear to be a manifestation of overall poor growth but mineral changes are greater than could be accounted for by poor growth alone. Depression of calcium and magnesium concentrations in radish shoots, and uptake of potassium in luxury amounts can somewhat be accounted for by the elevated levels of ammonia in the soil that occur when nitrification inhibitors are used, as past studies have shown these mineral aberrations in radish shoots when the plants were grown with ammonium-type fertilizer (Torrey et al., 1982). These results for K and Ca were also seen in cucumbers grown with N-Serve (Zawistowska et al., 1978).

Ammonium ions compete strongly with magnesium ions for uptake by plants (Mulder, 1950). This competition may be a direct result of the ammonium ions or it may be a function of the hydrogen ions released when NH_4^+ is assimilated. Depressed concentrations of magnesium in radish shoots may also be the result of large amounts of potassium made available when ammonium ions displace the potassium ions from the exchangeable soil sites. Excess potassium in soil solution depresses magnesium concentration in apple leaves (Mulder, 1950).

Likewise, depression of calcium concentrations in radish shoots may be accounted for, in part by excess ammonium ions in the soil solution. Many cations compete with calcium in

the growing medium, and the exchangeable binding sites on inorganic soil colloids are not very specific for calcium ions. Two hydrogen ions can displace one calcium ion from soil sites. These hydrogen ions may come from ammonium in the soil when it is converted to nitrate.

Whereas excess ammonium in the soil can partially account for depressed magnesium levels in soil solution and therefore in shoot tissue, the presence of ammonium in the soil causes more calcium to be found in soil solution, not less. Russell (1973) found that for every 100 kg of $(NH_4)_2SO_4$ added to the soil 45 kg calcium were leached out in drainage water. Depressed calcium concentrations in radish shoot tissue cannot be explained simply by soil chemistry as a result of ammonium nutrition, as much calcium is available to the plant. Researchers in the past (Barker, unpublished data) have noted lowered calcium concentration in foliage with plants grown in soil even when high amounts of calcium were supplied. When nitrification was inhibited potassium concentrations in radish shoots were much higher than for plants grown with no inhibitor, and one reason for this may be the higher levels of ammonium in the soil. Ammonium ions can displace potassium ions on soil colloids allowing for more potassium to occur in solution, and thereby allowing for more accumulation in radish shoots. Also, when calcium or magnesium uptake is low, competition with potassium uptake may be lowered, resulting in an increased potassium concentration in the radish foliage.

However, increased potassium concentration may be a result of a lessened "dilution effect", as the fresh and dry weights of radish roots and shoots were considerably lower when the plants were grown with an inhibitor.

As inhibitor levels were increased from the "25% treatment levels" to the "100% treatment levels" in Experiment Two, soil ammonium increased and soil nitrate-N decreased significantly. However calcium magnesium and potassium concentrations in the shoots remained at about the same levels for "25% treatment levels" as for "100% treatment levels". Therefore, as soil ammonium increased as more inhibitor was applied, mineral concentrations in foliage were not further affected as would be expected if soil ammonium alone was the cause for the mineral changes in the foliage.

Furthermore, soil ammonium in pots with inhibitors ranged from twice to 10 times the amount found in soil with no inhibitor (33 to 170 mg/ 1200 g soil (27 to 142 ppm) compared to 17 mg/1200 g soil (14 ppm)). These levels of soil ammonium far exceed the 24 ppm NH_4+ that is considered to be a high level of ammonium (Nicklow, 1981). However the plants grown with Dexon had all the appearances of normal plants in spite of the high levels of soil ammonium, 27.7 to 53ppm or 33.3 mg to 63.6 mg NH_4-N/pot (see Illustration 3). Plants grown with N-Serve and Truban appeared to have twisted roots and cupped leaves that were pale with distinctive yellow edges, and plants were spindly and stunted overall. These effects in appearance

were more pronounced at "100% treatment levels" of N-Serve and Truban despite no significant differences between the soil levels of ammonium at "25% treatment levels" to "100% treatment levels" of N-Serve and Truban. If soil ammonium alone were to account for the visual appearance of the twisted and cupped leaves, all of the plants grown with inhibitors should have exhibited this appearance, and plants grown with Truban should have been the most affected visually as those soils contained the most ammonium. This was not seen, as plants grown with Dexon appeared normal, and those grown with N-Serve exhibited the most severe visual symptoms. Therefore, I suggest the mineral content changes of radish foliage due to soil ammonium are occurring at as low as the "25% treatment levels", and because the mineral concentration changes do not further increase as soil ammonium increases but visual symptoms worsen, I also suggest that there is an effect of the inhibitors themselves.

N-Serve and Truban both belong to a family of chemicals known as trichloromethyl compounds (see Table 2). Dexon is a benzene-type chemical and, while it affects nitrification and mineral content, it does not affect appearance of the plants (see Illustration 3). The two trichloromethyl inhibitors, when applied to the soil result in radishes that appear to have symptoms of growth regulator effects similar to symptoms seen when excess auxins are present (Zawistowska et al., 1979). One ppm active ingredient of N-Serve in-

fluenced auxin-like activity in black locust seedlings (Lynd et al., 1966). This activity was manifested as severe leaf curling and twisting of stems. Auxins affect regulation of abscission, parthenocarpic fruit-set, rooting, stimulation of growth by cell division, and root formation (Eisinger and Morré, 1971) and auxins stimulate polarized shoot cell elongation (Morré and Key, 1967). Growing point necrosis appeared in the radishes grown with trichloromethyl compounds and growing point necrosis has been related in the past to auxin accumulation (Coke and Whittington, 1968). There are at least two possible explanations at present for accumulation of auxin. Boron may protect the indole acetic acid (IAA) oxidase system by complexing with inhibitors of IAA oxidase (Crisp et al., 1976). Perhaps it is a boron deficiency that occurs when trichloromethyl-type inhibitors are used. In the plant, manganese activates IAA oxidase (Mumford et al., 1962, Taylor et al., 1968, Morgan et al., 1966), and a deficiency of manganese would be expected to result in accumulation of auxins; however, analysis of radish foliage showed more manganese in the plants grown with nitrification inhibitors than in plants grown with none (see Table 8).

To further test my theory of trichloromethyl toxicity, I designed Experiment Three where I subjected radish plants to a trichloromethyl compound (N-Serve) without ammonium nutrition and to ammonium nutrition without a trichloromethyl compound present. In this experiment, it became evident that

while both compounds affect the mineral nutrition of radish foliage in similar ways (Ca, Mg, and K content changes in shoots), the two compounds produce markedly different visual symptoms. Excessive ammonium nutrition results in characteristic ammonium toxicity symptoms where root and shoot growth are adversely affected (Maynard and Barker, 1969). Ammonium toxicity causes chlorophyll to disappear and necrosis appears on leaf tissue, roots become corky and shortened (Barker et al., 1966). N-Serve and Truban in the soil produces plants which have symptoms of excess auxins. The symptoms occurred in the absence of excess ammonium and were more pronounced with organic fertilizers whan with the ammonium sulfate fertilizer. However, magnesium and calcium concentrations in radish foliage were the same for plants grown with ammonium sulfate and N-Serve as it was for those grown on Milorganite and N-Serve; potassium levels were higher in the plants grown with Milorganite than in those grown with ammonium sulfate. Ammonium levels in the soil were also higher, perhaps accounting for the increased uptake of potassium. Again a simple ammonium toxicity effect may be suggested if one considers only the ammonium and potassium data. However, the calcium and magnesium results suggest that deficiency of calcium or magnesium is not the sole cause for the increased growing point problems seen in plants grown with organic nitrogen sources. The auxin-like effects were so much more prominent on the plants grown with organic

fertilizers that a relationship between the symptoms and the nitrogen source may be suggested. Perhaps the Milorganite interacts with the nitrification inhibitors to affect nutrient levels in the foliage to such an extent as to inhibit IAA oxidase. At this time this can only be postulated, and more experiments should be designed to further study effects of nitrification inhibitors as influenced by availability of nutrients such as boron. Another study could be designed to further examine the role of the trichloromethyl family of compounds as plant toxins, and this family of compounds might interact with organic fertilizers and nutrient availability in such a way as to cause a compounded effect.

C H A P T E R V

CONCLUSION

N-Serve and Truban are effective nitrification inhibitors when nitrare-N concentration in radish shoots is used as an assay. When ammonium and nitrate-N contents of soil are used as an indicator of nitrification inhibition, N-Serve, Truban and Dexon were found to be effective. N-Serve, truban and Dexon depress calcium and magnesium concentrations and enhance manganese and potassium concentrations in radish foliage. N-Serve, Truban and Dexon applications increased soil ammonium content and decreased soil nitrate-N contentn relative to controls. Ammonium nutrition can account for some of the changes in mineral concentrations in the radish shoots. However, distinctive visual symptoms appear when radish plants are grown with N-Serve and Truban, two inhibitors that are trichloromethyl-type compounds, and these visual symptoms do not appear when radish plants are grown in soil amended with Dexon, a benzene-type compound, even when the ammonium content of soil was high. The visual symptoms are similar to the symptoms seen when plants were exposed to excess auxins (twisted, chlorotic leaves that are cupped in shape). These symptoms are more severe when Milorganite, an organic source of nitrogen, was the N source. It may be suggested, from data presented here, that trichloromethyl-type inhibitors exhibit growth regulator effects seperate from the effects of high concentrations of soil ammonium and that

these grown regulator effects are exacerbated by the use of an organic fertilizer.

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